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SHORT COMMUNICATION

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α/β- and γ/δ-T-cell-receptor-positive lymphocytes in healthy and inflamed human conjunctiva

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Abstract ● Background: The possible existence and distribution patterns of α/β- and γ/δ-TCR$^+$ cells, which are important constituents of immune surveillance and act via the CD3$^+$ cell complex have not yet been elucidated in the healthy and inflamed conjunctiva. ● Materials and methods: Paraffin-embedded conjunctival specimens included 18 from 18 patients with ocular cicatricial pemphigoid (OCP), 20 from 20 healthy controls, 6 from 6 patients with lye burns, and 6 from 2 patients with Stevens-Johnson syndrome; all were worked up by histology and immunohistochemistry. ● Results: α/β-TCR$^+$ cells were visualized in the conjunctival epithelium and stroma of healthy persons, OCP, lye burns and Stevens-Johnson syndrome. α/β-TCR$^+$ cells and a small number of γ/δ-TCR$^+$ cells were observed in the corneal epithelium and stroma of patients who have failing corneal grafts. After ileal mucosa transplantation to the epibulbar conjunctiva, membrane staining changes to nuclear and cytoplasmic staining. Treatment with systemic cytotoxic drugs abolishes all α/β-TCR$^+$ and γ/δ-TCR$^+$ cells. ● Conclusions: α/β-TCR$^+$ cells can be found in the non-infected epithelium and stroma of the healthy and inflamed (OCP, lye burns, and Stevens-Johnson syndrome) conjunctiva, as well as in the corneal epithelium and stroma of failing corneal grafts, whereas γ/δ-TCR$^+$ cells are absent. A small number of γ/δ-TCR$^+$ cells are present in the corneal stroma and adjacent conjunctival epithelium of patients with chronic corneal graft rejection or after transplantation of gut tissue. Further investigations may establish the role, if any, of these T-cell subsets in immune surveillance of the non-infected outer eye and in corneal graft rejection.

Introduction

The conjunctiva is an immunologically very reactive tissue. CD4$^+$ and CD8$^+$ T-cells are distributed in a specific pattern in the conjunctiva and fornix and have long been known to play an important role in the defense mechanisms of the outer eye. α/β-T-cell-receptor-positive (TCR$^+$) cells represent the majority of CD4$^+$ and CD8$^+$ T-cells in the lymphoid organs. In peripheral tissues and in inflammatory conditions, both α/β- and γ/δ-TCR$^+$ cells often express the CD3$^+$ marker and have been linked to the recognition of foreign peptides in the context of self-MHC complex molecules [12]. γ/δ-TCR$^+$CD3$^+$ cells account for only 1–5% of peripheral blood T-cells, but they have been described to be evenly distributed in lymphoid organs and in skin- and gut-associated lymphoid tissues. Different subsets of γ/δ−TCR$^+$CD3$^+$ cells are able to express cytotoxic activity, recognize heat-shock proteins, are involved in the defense of intracellular (myco)bacteria, and may play a role in autoimmune diseases and chronic allograft rejection and take part in lymphocytic infiltration of tumors [1].

γ/δ-TCR$^+$CD3$^+$ cells have been suggested to mediate immunologic surveillance of epithelia against transformed, infected, or autoimmunologically altered epithelial cells [3, 10, 14].

The presumed existence, distribution patterns, and possible roles of α/β- and γ/δ-TCR$^+$ cells in the healthy and diseased conjunctiva have not yet been elucidated. Therefore, we studied the distribution of α/β- and γ/δ−TCR$^+$ cells in healthy and non-infectious inflammatory disorders of the conjunctiva such as ocular cicatricial pemphigoid (OCP), Stevens-Johnson syndrome, and lye burns.

Materials and methods

Specimens

The conjunctival specimens used in this study were obtained from the Eye Pathology Laboratory of the University Eye Hospital Eppendorf. All of them were paraffin-embedded. Prior to biopsy, the patients had not been treated with specific eye ointment or drops for at least 1 week. Biopsies had been taken from bulbar nasal conjunctival sites, essentially where pathologically altered tissues were suspected.

Eighteen samples from 18 patients with OCP diagnosed between 1977 and 1994 were found to be appropriate for histopathological work-up and were compared to 20 samples of healthy conjunctival tissues taken as biopsy specimens during cataract surgery from otherwise asymptomatic patients.

Six samples from six patients with lye burns and six samples from two patients with Stevens-Johnson syndrome were also stained for T-cell subsets.

One each of the OCP, lye burns, and Stevens-Johnson syndrome specimens was noted to have suffered from a chronic corneal graft rejection.

Histology

The specimens were conventionally stained with hematoxylin and eosin, periodic acid-Schiff, and Alcian blue and were observed by light microscopy.

Immunohistochemistry

Specimens were prepared for immunohistochemistry using an antigen retrieval system. In brief, deparaffinized specimens were dehydrated, washed for 5 min in distilled water, and incubated in a microwave oven at 600 W for 3 min with prior application of antigen retrieval glyca solution. The probes were then washed and put into phosphate buffered saline (PBS, pH 7.4). Immunohistochemical identification of α/β- and γ/δ-TCR$^+$ cells was performed using commercially available monoclonal antibodies (anti-TCRδ1, anti-CrγM1, anti-Pan-TCR-α/β; T-Cell Diagnostics, Cambridge, Mass.) and the Super Sensitive detection system (BioGenex, San Ramon, Calif.). In brief, the primary antibody was applied to the slides, incubated for 30 min at room temperature, and washed in PBS for 5 min. Biotinylized link antibody was added and incubated for 20 min at room temperature. After washing in PBS for 5 min, the substrate consisting of alkaline phosphatase and fast red was applied to the slides and incubated for 7 min at 37°C, than washed in PBS for 5 min. Staining was performed with Mayer's hematoxylin and eosin and embedded with Aquatex. Specificity of the reaction was controlled using negative controls with mouse non-immune serum, and non-specific staining of the primary antibody was excluded with a negative control serum.

The slides were examined by light microscopy in a prospective fashion without knowledge of the disease entity. The density of infiltrating α/β- and γ/δ-TCR$^+$ cells was graded on a four-point scala (0 = no cells stained, 1 = <10 stained cells, 2 = 10–20 stained cells, 3 = >20 stained cells per high-power field).

Results

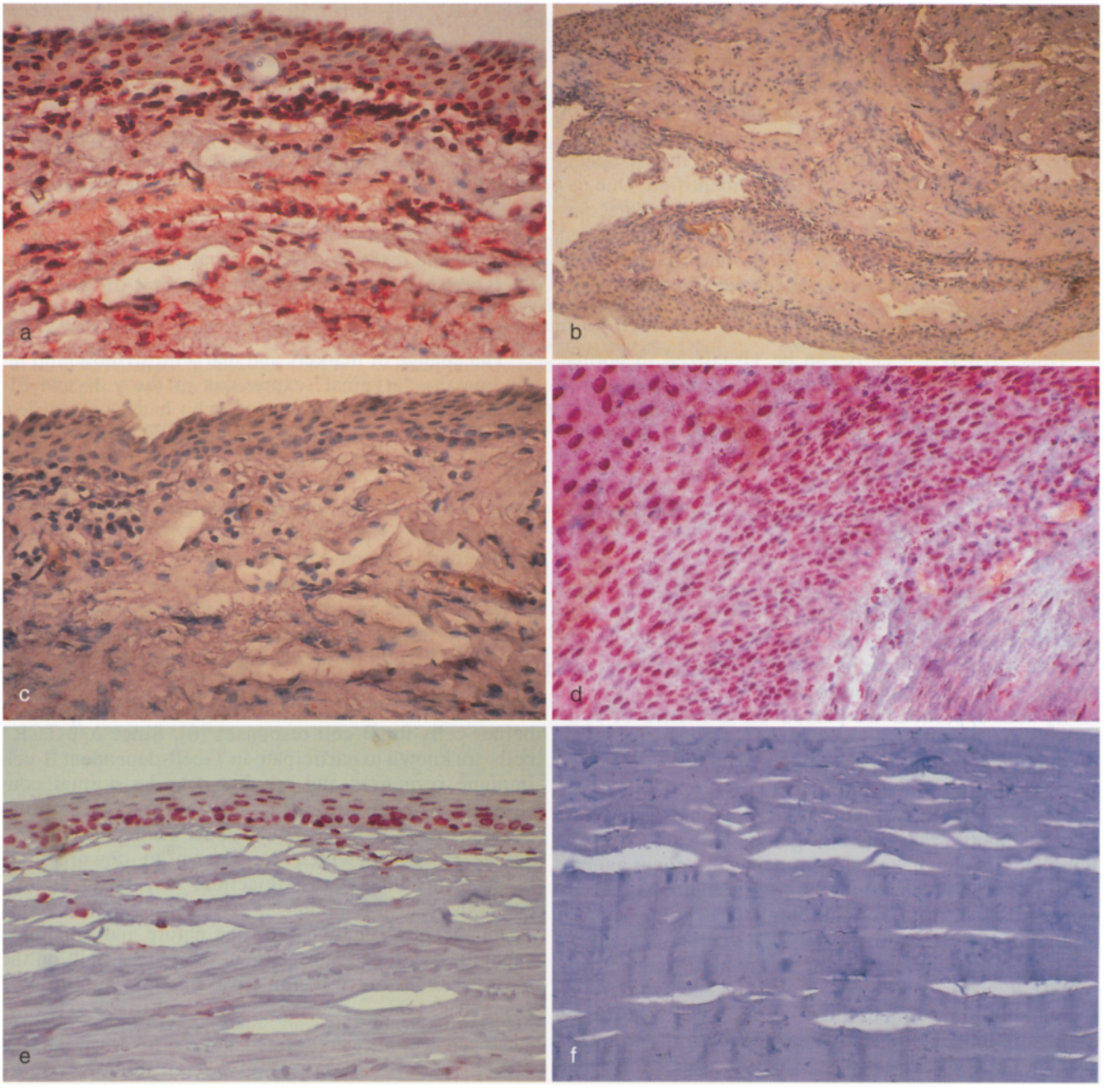
Healthy persons

All of the 20 conjunctival specimens from healthy persons had a normal epithelium, basal cell membrane, vascularized stroma, and amount of goblet cells. They all exhibited grade 1–2 α/β-TCR$^+$ cell infiltration in the epithelium and grade 1–2 in the stroma. None of these specimens showed any γ1- or δ1-positive reactions (Fig. 1a–c).

Ocular cicatricial pemphigoid

Each of the 18 conjunctival specimens from OCP patients exhibited the typical features of scarring in the conjunctiva and subconjunctivally, epithelial keratinization accompanied by a complete loss of goblet cells, and pronounced lymphocytic infiltration. Immunohistochemically, 15 of 18 specimens showed grade 2–3 staining for α/β-TCR$^+$ cells in the epithelium and stroma. Three specimens from patients who had taken cytotoxic drugs for control of disease did not stain with either α/β- nor γ/δ-TCR$^+$. In three specimens of patients who also had suffered from a chronic corneal allograft rejection, staining for α/β-TCR$^+$ cells (Fig. 1d) was grade 3 that for and γ/δ-TCR$^+$ cells was grade 1–2. γ/δ-TCR$^+$CD3$^+$ cell staining in these cases was primarily located in the epithelium. These conjunctival infiltration features were comparable to those of the cornea (Fig. 1e, f).

Fig. 1 **a** Immunohistochemical staining of epithelial and stromal T-cells expressing the α/β-receptor with an antibody anti-pan-TCR-α/β in the biopsy specimen of a healthy uninflamed conjunctiva. **b** No staining of cells with anti-CrγM1 in the biopsy specimen of a healthy uninflamed conjunctiva. **c** No staining of cells with anti-TCRδ1 in the biopsy specimen of a healthy uninflamed conjunctiva. **d** Strong staining of densely infiltrating epithelial and stromal T-cells expressing the α/β-receptor (anti-pan-TCR-α/β) in the limbal conjunctiva adjacent to a failing corneal graft in ocular cicatricial pemphigoid. **e** Immunohistochemical staining of mainly epithelial and some stromal T-cells expressing the α/β-receptor (anti-pan-TCR-α/β) in the corneal specimen of a chronic failing graft in ocular cicatricial pemphigoid. **f** Immunohistochemical staining of only one stromal T-cell (at the 12 o'clock position) expressing the γ1-receptor (anti-TCRγ1) in the corneal specimen of a chronic failing graft in ocular cicatricial pemphigoid (for details of methods, see text)



Stevens-Johnson syndrome

Each of the two conjunctival specimens from patients with Stevens-Johnson syndrome featured extensive scarring in the conjunctiva and subconjunctivally, focal ulceration and hyperplasia of the epithelium, keratinization accompanied by a complete loss of goblet cells, and some lymphocytic infiltration. One specimen from one patient who had already received a tectonic keratoplasty (with chronic failure) showed grade 2–3 membrane staining for α/β- and grade 1–2 membrane staining for γ/δ-TCR$^+$ cells in the conjunctival epithelium and the stroma. After the patient had received an ileal mucosal graft the staining pattern of the cells changed. It was no longer membrane-associated; α/β-TCR$^+$CD3$^+$ cell staining was nuclear, and γ/δ-TCR$^+$ cell staining was cytoplasmic. The conjunctival specimen of the second patient, taken prior to nasal mucosa transplantation, revealed α/β-TCR$^+$ cell membrane staining. After nasal mucosa grafting additional γ/δ-TCR$^+$CD3$^+$ cell staining (grade 1–2) was observed.

Lye burns

Each of the six conjunctival specimens from lye burns featured extensive scarring in the conjunctiva and subconjunctivally, keratinization and partial hyperplasia of the epithelium accompanied by a complete loss of goblet cells, and some lymphocytic infiltration. In five specimens, only α/β-TCR$^+$ cells could be identified. One patient who had received a corneal graft which had decompensated due to elevated intraocular pressure exhibited α/β-TCR$^+$, but no γ/δ-TCR$^+$ cell staining. However, when an ileal mucosa graft had been performed, the conjunctival biopsy specimen revealed grade 2–3 α/β- and γ/δ-TCR$^+$CD3$^+$ cell staining.

Thus, α/β-TCR$^+$ cells can be visualized in normal healthy conjunctival epithelium and stroma and in OCP, lye burns, and Stevens-Johnson syndrome. Additional γ/δ-TCR$^+$ cell staining was seen in patients who had received a corneal graft.

Discussion

In this study we used the antibodies anti-TCRδ1, anti-CrγM1, anti-TCRδ1, and anti-pan-TCR-α/β, since γ1 and δ1 genes coding for the heterodimer T-cell receptor γ/δ are known to home to epithelia and peripheral tissues, and α/β to lymphoid organs.

By this approach, we proved the existence of α/β-TCR$^+$ cells and the absence of $\gamma/\delta-$TCR$^+$ cells in the conjunctival epithelium and stroma of healthy persons as well as in non-infected inflamed tissue. These cells home primarily to lymphoid organs and are believed to play a major role in the generation of cytotoxic lymphocytes and in the defense against infectious agents, since they can induce high-affinity interleukin-2 receptor expression, proliferation, cytolytic activity, and secretion of lymphokines via the CD3$^+$-cell complex.

Acute graft rejection is due to the high frequency of α/β-TCR$^+$ cells, which recognize allogeneic major histocompatibility complex proteins. γ/δ-TCR$^+$ cells are known to downregulate allograft reactions [8, 18]. This fact may represent the reason for the observation that γ/δ-TCR$^+$ cells infiltrate tissues with failing corneal grafts due to chronic rejection.

Although tissue-associated γ/δ-TCR$^+$CD3$^+$ cells have been found to play a role in several diseases presumed to be autoimmune, including sarcoidosis [2, 15], rheumatoid arthritis [4, 5, 10, 11, 16], systemic lupus erythematosus [13], celiac disease [17], multiple sclerosis [8], and atopic dermatitis [7], we have not been able to detect these cells in inflamed conjunctival tissues of OCP or in Stevens-Johnson syndrome, considered as an autoimmune diseases. However, patients suffering from OCP who had been treated with cytotoxic drugs up to 1 week prior to biopsy did not reveal either α/β- or γ/δ-TCR$^+$ cells in the epithelium or stroma.

The change of markers and marker distributionm, including the "atypical" expression of the γ 1- and δ1-TCR$^+$ cells in the two patients who had received ileal mucosa grafts, can be well explained by earlier reports on the observation of γ/δ-TCR$^+$CD3$^+$ cells in the gut in health and disease [19]. These gut-associated cells obviously persist in the conjunctiva for several months after heterotopic transplantation.

The data presented in this study of T-cell subsets in healthy and inflamed conjunctiva add to our knowledge of cellular mechanisms of the conjunctiva-associated lymphoid tissue. The principal T-cell subset known to stimulate B-cells to secrete antibody against T-cell dependent antigens carries the α/β-TCR$^+$ marker [6, 9]. γ/δ-TCR$^+$CD3$^+$ cells do not seem to play a major role as helper cells for B-cell responses [8]. Since α/β-TCR$^+$ cells are known to participate in T-cell-dependent B-cell immune responses and are found to be normal constituents of the healthy outer eye, they may play an important role in defense mechanisms of the conjunctiva. γ/δ-TCR$^+$ cells, which are known to downregulate mechanisms of allograft rejection, may be of significance in corneal rejection.

Further investigations into the nature and functional capacities of T-cell subsets may shed more light on the basic mechanisms of conjunctival immune responses.

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